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ONTOGENY OF EGF RECEPTORS IN THE HUMAN GUT

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1. ABSTRACT

Epidermal growth factor and related substances mediate their effects on epithelial cells through binding to high-affinity receptors (EGF-R) at their basolateral surface and it is hypothesized that this growth factor system play a major role in gut morphogenesis and maintenance. The current review emphasizes on analyzing the expression and the biochemical characteristics of EGF-R in human fetal gut segments and correlating the biological actions of EGF-R ligands. They appear to be primarily involved in the local regulation of epithelial cell proliferation in which EGF-R are abundant. Alternatively, EGF-R ligands exert some precocious maturative effects by increasing intestinal lactase activity and decreasing brush border hydrolases in colon while they down modulate the expression of segment-specific markers of terminal differentiation such as sucrase, trehalase and glucoamylase in the intestine and chief cell lipase in the stomach. Such effects are consistent with the identification of receptors at the surface of all epithelial cell types, illustrating the modulatory role of EGF on differentiated gut epithelial cells. Comparison with animal models illustrates similar biochemical properties of

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receptors and underlines physiological aspects specific to human gut development. The relevance for ligand heterogeneity is also discussed and tentatively associated with different delivery pathways or physiological responses.

2. INTRODUCTION

Epidermal growth factor or EGF is one of the best characterized growth factors that was initially isolated by Stanley Cohen as a contaminating material in nerve growth factor preparations promoting the growth of embryonic neurons (1,2). Early on, it was found to be abundant in some body fluids i.e. saliva, serum, milk and urine (3-6). Accordingly, radioimmunoassay analysis in conjunction with immunohistochemical studies demonstrated that the submaxillary salivary glands, duodenal Brunner's glands, mammary glands and metanephric kidneys represented major sources of the EGF peptide (7-9). EGF-related material (transforming growth factor-alpha or TGFalpha) was also extracted from the amniotic fluid (0.25-4.3 ng/ml; refs 9-11) and found to be produced by extraembryonic membranes (trophoectoderm, placenta) in animals and humans (12-15) although a fraction of this material is likely to derive from developing kidneys (9). Taken altogether, these findings lead to the hypothesis that EGF or related forms released from these sources could be involved in embryonic development and gut morphogenesis or the maintenance of gut tissues in the adult. A major breakthrough was then achieved when EGF mRNA transcripts and EGF-like substance/ activity were detected in lower amounts in various tissues (16-18). Thus, it was proposed that the growth factor could act *in vivo* as a local paracrine factor in addition to its role as a long-distance acting hormone.

Concerning the characterization of the biological action of EGF in tissues, pioneering studies (19,20) using subcutaneous injection protocols in neonatal mice helped to recognize the maturing epidermis and its appendages as important targets of EGF action. It was initially observed that the factor accelerated eyelid opening and incisor eruption (thus accounting for the 'tooth lid factor' denomination used in the early 1960's). Ulterior experiments showed that EGF was also able to stimulate hair growth and epidermal cell proliferation and keratinization (21). The mitogenic effect of EGF was then rigourously studied *in vitro* on fibroblasts (22-25) where the predicted presence of EGF surface receptors was verified for the first time using iodinated (^{125}I)EGF binding techniques (24,26). As will be discussed further, the presence of surface binding sites was also demonstrated in mesodermal and endodermal derivatives, especially in gut epithelial compartments, and the gene encoding the EGF-R protein in rodents and man was successfully cloned and sequenced (see reviews 27-29).

In 1983, an oncogenic protein, termed v-*erb*B1, was identified as a product of avian erythroblastosis virus (30) which causes the abnormal presence of immature blood cells (erythroblasts) into systemic circulation in chicken. Viral *erb*B1 in fact represents a truncated and constitutively-active form of the EGF-R (30); a cellular homolog oncogene named c-*erb*B1 was identified in embryonic and tumor cells in rodents (31), and then found to be identical to the mouse EGF-R. Such discoveries and biochemical/immunological data from pathological studies permitted to establish a functional relationship between the expression of EGF-R and the

initiation of cancer, as proposed for the gut in particular, where the relative abundance of EGF-R serves as a prognostic factor in esophageal, gastric and colon tumorigenesis (32-34). Enhanced expression of EGF-R may also be a marker for the increased risk of colon cancer in inflammatory bowel disease (35).

In the following years, the expression pattern, the mechanism of activation, the recycling through the endocytic pathway as well as the involvement of EGF-R in the biological action of EGF were extensively studied in normal tissues and cultured cells originating from animals and man. As examplified in basic reviews (2,36-40) integrating all aspects of EGF/EGF-R biochemistry and physiology, the expression and function of this growth factor system seem to be primarily associated with the involvement of EGF as a competence growth factor stimulating the survival, the migration and the mitosis of stem cells and pre-differentiated cells, including those of the gastrointestinal tract giving rise to mature epithelial cells. The more recent development of gene knock-out and promoter engineering strategies which enable the generation of mouse lineages carrying either EGF/TGFalpha/EGF-R genes inactivated by defective mutations or genes which expression is forced by an active promoter (mutations integrated in the whole organism or targeted to specific organs) indeed supports the latter concept. Overexpression of the TGFalpha transgene (41-46) primarily alters epithelial proliferative compartments and ultimately leads to a desequilibrium between pre-differentiated and differentiated cell populations: hyperplasia and rarefication of differentiated phenotypes. Alternatively, null mutations of TGFalpha/EGF-R genes (47-50) reveal that they exert a complex function since their deficiencies not only modify cell proliferation but perturb a wide range of developmental activities (branching morphogenesis, epithelial cell differentiation or maturation, skin architecture). In accordance with the latter observations, other pioneering studies suggest that EGF itself or EGF acting in synergism with other growth factors plays a role in the differentiation of specific cell species such as keratinocytes (as mentioned above; ref.19-21), palatal cells (51), surfactant-secreting pneumocytes (52,53), cervical cells (54), nasal epithelial cells (55), ameloblasts/odontoblasts (56), trophoblasts (57) as well as enterocytes (58-60). These observations jointly reinforces the physiological importance of EGF and EGF-related molecules as cell inducers and stresses the complexity of regulatory processes involved in organogenesis and tissue maintenance. In this context, a growth factor system may play an epigenetic role by stimulating distinct functions in different cell types at specific developmental stages when the ordered sequence of survival, proliferation and differentiation is normally progressing. The following sections describe the general properties of the EGF-R as well as its expression pattern and biological significance in specific segments of the human fetal gastrointestinal tract.

3. IDENTIFICATION AND BIOCHEMISTRY OF EGF RECEPTOR

3.1. Gene and protein structure

The nucleotide sequence of the EGF-R, which is termed HER-1 in human (61), predicts a 1186-1210 aa backbone with a molecular weight of 134-135 kilodaltons (kD)(see reviews 36,38). The mature polypeptide is glycosylated at 11-12 asparagine residues, its molecular mass is estimated to ~170 kilodaltons in various tissues and it is characterized by intrinsic tyrosine kinase activity (62-65). In addition, the

extracellular region contains 51 cysteine residues which are concentrated in two domains that cooperate to form a high affinity EGF binding site (66,67; see figure 1). *Drosophila* and *Caenorhabditis* EGF-R homologs contain the same functional domains (68,69) thus revealing a high degree of evolutionary conservation. Although a single human EGF-R gene exists, two spliced variants were revealed in A431 cells (64,70,71) which served in many laboratories as a model for studying EGF-R expression and subcellular trafficking. The physiological significance of this process remains uncertain however since this epidermoid carcinoma cell line produces aberrant forms of the receptor which are absent in nonmalignant tissues (64,72,73). Only a few data concerning the possible existence of alternative splicing mechanism in human carcinomas (71) and developing kidney (74) were obtained in the past. Presumably, this subject has not retained much attention because the respective contributions of 10- and 5.6-kb transcripts for protein expression have not been examined. Nonetheless the recent discovery of a new spliced mRNA species in human placenta with a lower number of base pairs (75) may lead the way to a new comprehension of post-transcriptional regulatory processes controlling the tertiary conformation and function of the EGF-R.

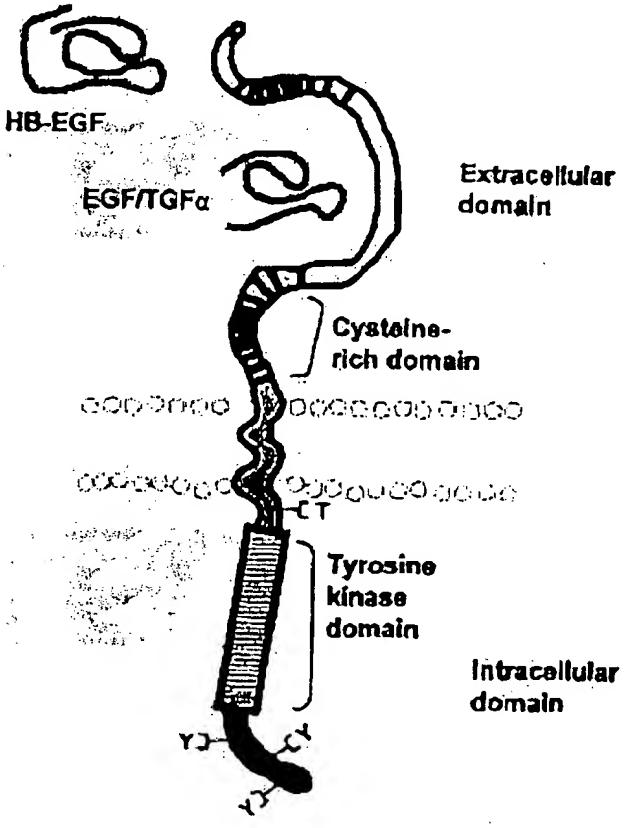


Figure 1. Tertiary structure and consensus motifs of the EGF-R and its common ligands, EGF, TGFalpha and HB-EGF. The extracellular portion of the receptor contains two domains enriched in cysteine residues (black bars). The intracellular portion contains 1) a catalytic domain with tyrosine kinase activity and 2) several phosphorylation sites at threonine (T) and tyrosine (Y) residues.

3.2. Mechanism of activation

Early studies have demonstrated that EGF binding to the extracellular domain of EGF-R stimulates its tyrosine kinase activity causing increased phosphorylation of protein substrates as well as autophosphorylation of tyrosine residues near the C-terminus region (76). Moreover, threonine phosphorylation of the EGF-R by protein kinase C reduces its binding affinity and negatively regulates tyrosine kinase activity (77). Indeed the functionality of EGF-R varies according to the cell status; high- and low-affinity binding sites were identified in many cell types (78,79) that could be the result of post-translational modifications (phosphorylation, interaction with other proteins)(80,81).

Biochemical data showed in the past that an orderly sequence of receptor dimerization, clustering and internalization is normally activated following EGF binding (82) allowing the cellular response and the endocytic recycling of EGF/EGF-R complexes, as for many receptor systems. In fact, the physical aggregation of multiple EGF/EGF-R complexes at the level of the plasma membrane appears to be required for subsequent activation of tyrosine kinase activity (83,84). Numerous studies have demonstrated over the years that the latter process leads 1) to activation of intracellular effectors associated to EGF-R and which are organized into multimeric complexes (src, ras, phospholipase C-γ , phosphatidylinositol 3-kinase, etc.) and 2) to various nuclear events required for mitogenesis, transformation and cell differentiation (reviewed in 85,86). Recent data even suggest that the receptor aggregation process as well as the optimal phosphorylation of associated effectors are operated through a cooperation with the integrin/matrix apparatus (87). This assumption remains to be verified specifically for the EGF/EGF-R system in the gut.

3.3. General distribution

Putative EGF-R were initially identified and characterized using ^{125}I-EGF binding techniques in a wide range of cell types. Radioautographic data soon established that they are preferentially localized on the plasma membrane of epithelial cells, and at their basolateral pole (reviews 36-40). Moreover their number is high in proliferative cells, neoplastic cells and carcinoma cell lines (27,88). In A431 cells for example, EGF-R are overexpressed by 20 to 50-fold compared to normal tissues (27,64,72). Using the same strategy, receptors were successfully detected in embryonic/fetal and extraembryonic tissues of rodents (36,38,40) where they generally increase in number during gestation while their affinity for the ligand EGF somewhat decreases (89). Also, when fetal organs are explanted and cultured with EGF, the incorporation of (^{3}H)thymidine into DNA is usually stimulated (90).

Then with the advance of immunological techniques, anti-EGF-R antibodies were produced that helped to confirm the precise localization of the protein at the tissular and subcellular levels. The general distribution of EGF-R in human fetal tissues (91-93) was found to be quite similar, as expected. In comparison with rodents however, the number of receptors tend to decrease before birth probably reflecting the more advanced degree of organ maturation that is achieved *in utero*. One interesting new discovery made on cultured isolated cells pertains to the concentration of EGF-R and associated effectors of signal transduction into adhesion plaques or cytoskeleton-membrane focal adhesions (94,95).

3.4. Ligands

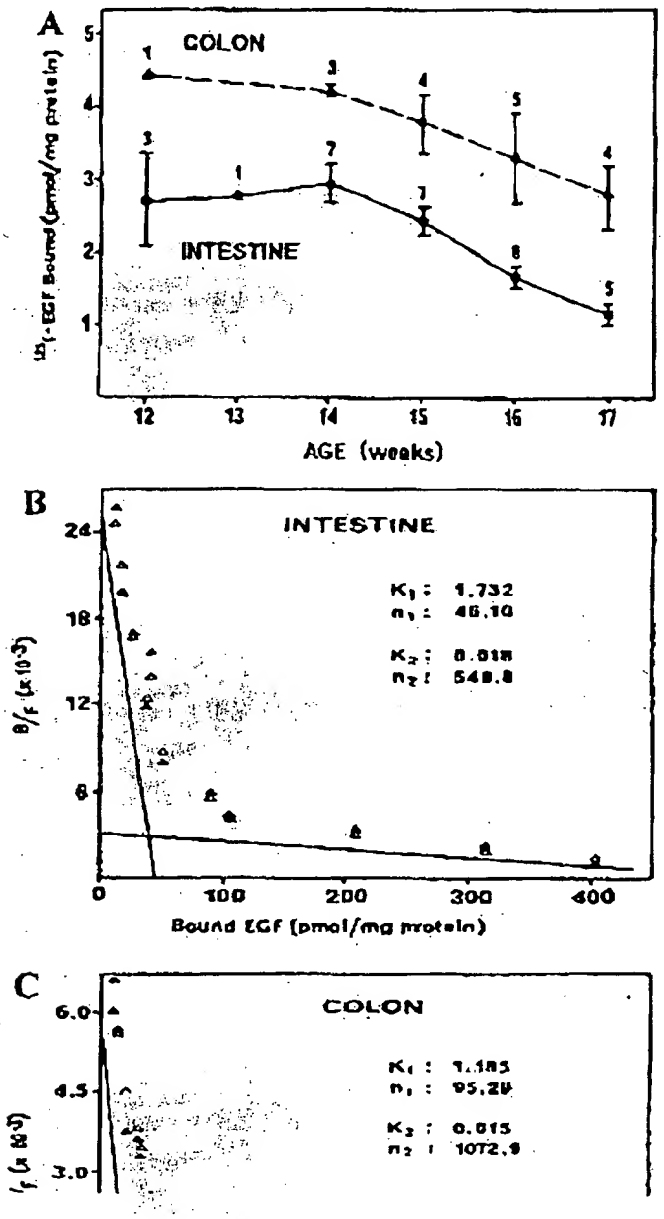
EGF is a 53 amino acid polypeptide, with a molecular weight of 6045 daltons, which possesses three intrachain disulfide bonds. It is the prototypical member of a family of growth factors that also includes human pancreatic secretory trypsin inhibitor (96), transforming growth factor-alpha (TGFalpha)(97,98), vaccinia growth factor (99,100), schwannoma-derived growth factor (SDGF) or amphiregulin (AR) (101,102), betacellulin (103), cripto-1 (CR-1)(104), heparin-binding EGF-like factor (HB-EGF)(105), the *neu* differentiation factor (NDF)(106), the heregulins (107), and two homeotic gene products, the *drosophila* Notch protein (108) and the lin-12 protein of the nematode *Caenorhabditis elegans* (109). EGF-related molecules derive from large membrane-bound precursors which are released by proteolytic cleavage (110). PreproEGF, for example, comprises 1217 aa, seven EGF-like repeats in addition to the mature EGF coding region (111,112), and a portion of the molecule exhibits limited homology with the low density lipoprotein (LDL) receptor, suggesting that the EGF precursor and the LDL receptor descended from a common ancestral transmembrane protein (113,114). TGFalpha derives from a shorter 160 aa precursor (115) and it exhibits 33-44% sequence homology with mouse or human EGF (12 residues are strictly conserved). Of interest, its discovery in the culture medium of established cell lines contributed to elaborate the autocrine hypothesis of cell growth control (116,117). In comparison, HB-EGF is characterized by a longer N-terminal arm with a specific structural motif that confers sensitivity to sulfated glycosaminoglycans and proteoglycans (118). All three peptides represent potent agonists of the EGF-R in several normal (non transformed) tissues and cell lines.

4. EXPRESSION IN HUMAN FETAL GUT SEGMENTS

4.1. Small intestine

The presence of EGF-R in human fetal small intestine was demonstrated for the first time in 1988 by binding measurements (119). In this study, classical ^{125}I-EGF binding procedures were performed at 22° C for 45 minutes on epithelial cell preparations that were obtained by manual shaking (in EDTA/NaCl solution) of everted segments of small intestine. For Scatchard analysis, cells were incubated with the iodinated ligand plus increasing concentrations of unlabelled EGF(10^{-11} to 10^{-7} M) and non-linear plots were analyzed according to the two-site model. The pattern of ^{125}I- EGF binding between 12 and 17 weeks (postfertilization) shows that binding was significantly higher (2.5-fold) in 12 to 14-week fetuses than in older fetuses, as illustrated in figure 2. In percentage terms, specific binding in youger fetuses represented more than 16.5% of labeled EGF/mg cell protein whereas 17-week-old specimens bound less than 6%. Low- and high-affinity binding sites were identified with association constants of $K_1 = 1.90 \pm 0.45 \times 10^{-9} M^{-1}$ and $K_2 = 0.033 \pm 0.016 \times 10^{-9} M^{-1}$ respectively. This binding was also specific; no cross-competition for EGF binding sites was observed with insulin, ACTH, thyroxine, hydrocortisone, IGF-1, IGF-2 or dexamethasone when they were added in excess concentration together with EGF. Quantitative autoradiography of ^{125}I-EGF binding (120) reveals

extensive accumulation in undifferentiated cells of the crypt and at the base of the villus, as well as in the inner circular layer of the muscularis externa bordering the submucosa (some labeling was detected in mesenchymal and vascular elements of the lamina propria). A gradient of silver grain density was clearly established along the crypt-villus axis towards the regions of high proliferative activity. Epithelial cells in the deep portion of the crypt showed the highest density (9.2 grains/μ m^2), which gradually decreased in the upper crypt (6.5) and the lower villus (3.9), with very little labeling in the upper third of the villus (0.4). The cellular distribution of silver grains in epithelial cells of the lower villus revealed a polarization of labeling in the basolateral infranuclear region (6.0 grains/μ m^2 *versus* 0.7 in the supranuclear/brush border compartment). The last observations support the hypothesis that EGF would access to its receptors on epithelial cells from the serosal side, not from the gut lumen, under normal non-pathological conditions. Confirming this assumption, the same study (120) demonstrated that labeled EGF could not access to its receptors when infused into the lumen of jejunal segments, either at 22° C or 4° C.



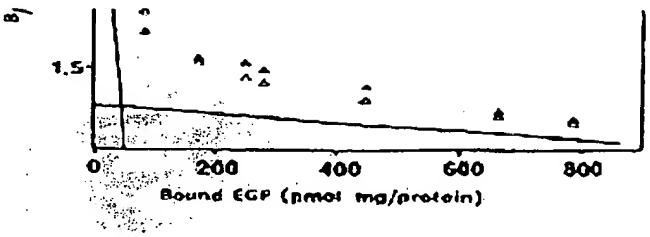


Figure 2. Developmental pattern (A) and Scatchard analyses (B,C) of iodinated EGF binding to epithelial cells isolated from human fetal small intestine and colon. Indices in graph A represent the number of specimens for each age (adapted from ref 119).

Of particular interest, the small intestine undergoes extensive morphogenesis between 8 and 12 weeks of gestation with the onset of villus and crypt formation and subsequent transformation from pseudostratified columnar to simple columnar epithelium (121). Labeling indices within this epithelium are also at their highest value between 8 and 10 weeks (approximately 26-30%) and decrease markedly during the next 4-6 weeks (122). It is thus during this critical period of morphogenesis and remodeling that the intestinal epithelium exhibits its highest EGF binding capabilities. Moreover, Poulsen and co-workers (9) have demonstrated the presence of immunoreactive EGF in intestinal Paneth cells of the 20-week-old fetus suggesting that this cell type represents an endogenous source of the peptide. Paneth cells normally appears at 11-12 weeks (122) which again correlates with the high number of EGF-R at this early age. The possibility that EGF from Paneth cells may influence the neibourghing undifferentiated cells has yet to be explored. In subsequent studies using the immunohistochemistry approach (123,124), the presence of EGF-R at the basolateral cellular pole, their consistent absence in brush border preparations and their rarefication with increasing age were verified in intestinal epithelium of fetuses, children and adults.

Two colon carcinoma cell lines, namely Caco-2 and HT-29, exhibit human enterocyte-like features in culture and are commonly used as *in vitro* models to study the role of putative regulators. The capacity of these cells to bind EGF has been demonstrated (125) as well as the involvement of an EGF-like factor in their autonomous growth (126,127) and migration behavior over laminin substrate (128). Recent data indeed show that antisens EGF-R expression has an antiproliferative effect on HT-29 cells (129). While both Caco-2 and HT-29 cells express a minor pool of EGF-R at their apical surface, only basolateral membrane stimulation with EGF increased tyrosine kinase activity and enhanced proliferation (130,131), in accordance with the proposed model of EGF action on epithelial cells. EGF not only stimulates DNA synthesis and proliferation of Caco-2 cells but reduces sucrase activity markedly, by affecting its processing in the endoplasmic reticulum and mRNA synthesis (132). The same study reveals that EGF maintains Caco-2 cells in a poorly-differentiated phenotype thus suggesting that the growth factor would act as a mitogen and a repressor of terminal differentiation. Interestingly, ulterior experiments showed that parental HT-29 and sublines lose their proliferative response to EGF ligands as they spontaneously differentiate in culture (133): they are mitogenic for undifferentiated cells but inhibit the growth of more differentiated cells. Changes in the signaling machinery rather than the modulation of EGF-R

expression appear to be involved in this process.

The study of EGF-R expression and ligand activity in primary cultures of human small intestinal cells still awaits the development of a suitable experimental system. Nonetheless, the biological effects of EGF have been carefully examined in organ cultures of fetal jejunum (11 to 14 week of gestation) (134). While the addition of exogenous EGF did not modify the morphology of intestinal explants, lactase activity was significantly increased and the rise in sucrase, trehalase and glucoamylase activities that normally occurs during culture was repressed in the presence of increasing concentrations of EGF. DNA synthesis and labeling index dropped drastically (within 24 hours of culture) in this model, thus recalling the response of differentiated cultured cell lines. These results clearly suggest that the influence of EGF on the regulation of small intestinal epithelium development depends upon the differentiation status of target cells.

4.2. Colon

Binding of ^{125}I-EGF to colonic epithelial cells isolated from 12 to 17-week fetuses was assessed using the same methodology and optimal conditions as for intestinal epithelial cells (119; figure 2). Compared to the small intestine, specific binding in the colon was substantially higher (1.4 to 2.4-times) at all ages and also decreased with advancing age (figure 2). High-affinity ($K_1 = 1.78 \pm 0.83 \times 10^{-9}$ M^{-1}) and low-affinity ($K_2 = 0.014 \pm 0.005 \times 10^{-9}$ M^{-1}) binding sites were similarly demonstrated by Scatchard plot. Their cellular distribution in smooth muscle fibers as well as in developing villi and crypts of the fetal colon was identical to that found in small intestine, being concentrated in regions of high proliferative activity and tritiated thymidine uptake while being absent from the brush border of villus cells (120).

The overall pattern of EGF binding in the human fetal colon is notheworthy since colonic epithelial cells exhibited even higher binding than their intestinal counterpart. This difference can be attributed partly to a greater concentration of EGF-R at the cell surface. Moreover, since the onset of villus formation and epithelial differentiation in the developing colon occurs 3-4 weeks after initiation of morphogenesis in the small intestine, there may exist a relation between the fact that levels of EGF binding in the colon at 17 weeks are equivalent to peak levels observed in the 12-14 week-old intestine (figure 2). It is also known that the expression of intestinal-type markers are down-regulated and that fetal colonic villi are transformed into adult-type colonic crypts before birth. Whether the expression and binding kinetics of EGF-R are modified during this ontogenic process, when epithelial cell proliferation contributes to tissue remodeling, remains to be determined.

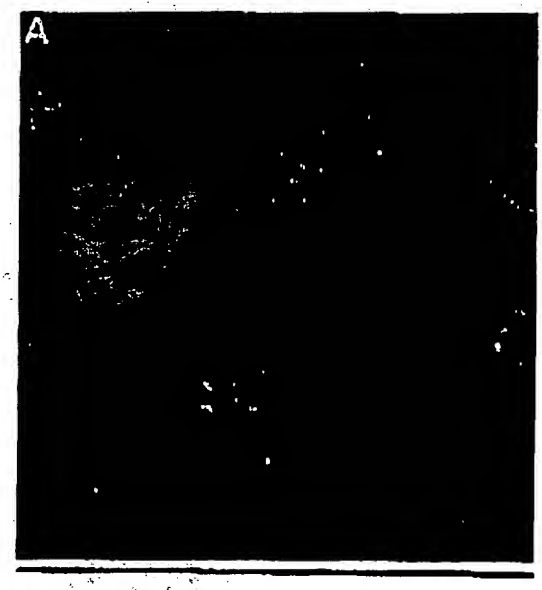
As mentioned previously for the small intestine, the influence of EGF was studied in organ cultures of human fetal colon (135) where it did not alter the overall morphology of explants. Biochemical data, however, suggest that EGF may participate in the maturation process (loss of villus markers) since it caused a decrease of DNA synthesis rates and brush border sucrase, maltase and alkaline phosphatase activity levels. Illustrating the significance and the specificity of EGF

action, hydrocortisone generated no effect in this model (135).

4.3. Stomach

The biological relevance of EGF action at the level of the stomach has been demonstrated early on. The molecule called urogastrone, an inhibitor of gastric acid secretion produced by the human kidney and present in urine, was proven to be identical to the mature EGF peptide (136). The presence of EGF-R in the adult gastric mucosa was also verified and their increased expression in foveolar and surface epithelial compartments (compared to the base of glands) supports a pivotal role for this growth factor system in the maintenance of gastric mucosal integrity (protection, healing)(137-139). However the developmental aspects of EGF-R physiology in the human fetal stomach were only studied recently.

The presence of the EGF-R immunoreactive protein was first suggested in a study analyzing its widespread expression in digestive tract epithelia and pancreatic tissues (123). Using indirect immunofluorescence and autoradiographic localization of ^{125}I-EGF binding sites, it was then demonstrated that the pattern of EGF-R expression in developing stomach significantly differed from the adult situation (140). In fact, EGF-R were detected as soon as 10-11 weeks of gestation when the gastric mucosa is lined by a stratified undifferentiated epithelium. At subsequent stages (12 to 20 weeks; figure 3), all epithelial cell types of the developing pit/gland structure (undifferentiated, mucous, endocrine, parietal and chief cells) were immunoreactive and the three major functional compartments e.g. the surface epithelium, the pit/neck region and the gland contained comparable numbers of binding sites. Thus, when compared to the adult stomach, it seems plausible that EGF-R and its ligands exert a broad range of developmental activities in the fetal organ due to their ubiquitous expression in all gastric epithelial cell species. As expected also, the cellular distribution of EGF binding was concentrated in the infranuclear region of epithelial cells (140). Reflecting the highly polarized localization of EGF-R and, possibly, the early compartmentalization of EGF-R expression in the pit/gland unit, an intensive immunostaining was seen at the basolateral pole of surface mucous cells in 20-week specimens (figure 3).



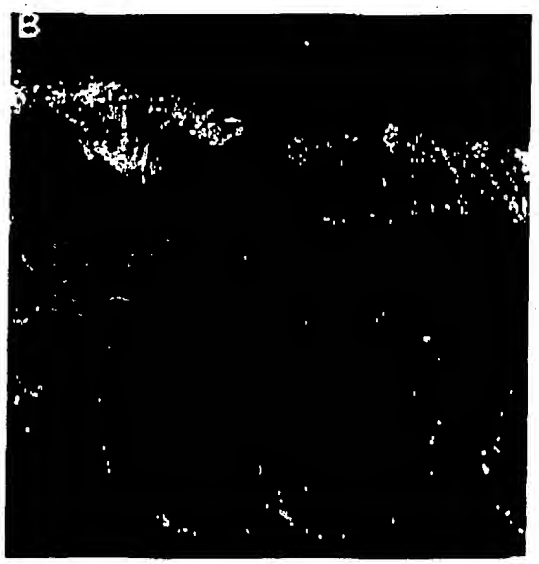


Figure 3. Localization of immunoreactive EGF-R protein in human fetal stomach mucosa at 12 (A) and 20 (B) weeks of gestation. Apical staining on surface cells represents non specific binding of antibody to mucus (from ref.140).

In the past, only a few studies were aimed at studying the role of the EGF/TGFalpha/EGF-R system in cultured human gastric tissues. One report (141) helped to confirm that EGF-R are present on epithelial cells isolated from adult stomach and showed that exogenous EGF stimulates the release of an angiogenic growth factor. Results obtained with human fetal gastric tissue maintained in organ culture (140) demonstrated that EGF regulates the proliferative and differentiative functions of the developing epithelium. The factor was able to stimulate DNA synthesis in mitogenically-competent cells and the incorporation of tritiated glucosamine into newly-synthesized glycoproteins (in mucous cells). Moreover, EGF down-regulated lipase activity in this model without affecting pepsin, two digestive enzymes co-localized in human glandular chief cells. The latter observations are consistent with the ubiquitous expression of EGF-R in all epithelial cells of the fetal gastric mucosa and further reinforce the assumption that associated ligands might control a variety of developmental activities contributing to the differentiation and/or the maintenance of gastric epithelial lineages. These results also emphasize the uncoupled regulatory process for lipase and pepsin, and enlighten the role of EGF in the modulation of lipolytic activity of the human gastric mucosa. Recently, it has been shown that EGF exerts its down-regulatory action on gastric lipase at the mRNA level (142).

5. COMPARISON WITH ANIMAL MODELS

On a general basis, the characteristics of EGF receptors in both human fetal intestinal and colonic epithelial cells in terms of binding kinetics, ligand specificity and binding affinities ($K_d = 10^{-10}$ to 10^{-9} M) are typical of those found in rodent intestinal epithelial cells using the same protocols (143-145). As seen for the human, the concentration of EGF-R was higher in mouse developing colon than in small intestine at the same postnatal age (146).

While there is a general agreement about the tissular localization of EGF-R and the growth-promoting effect of EGF-related factors on the adult gastrointestinal tract of several species, discrepancies between humans and rodents have been reported regarding its implication in the functional differentiation of intestinal epithelia during ontogeny (refer to comprehensive reviews 147-149). For example, EGF promotes in organ culture the maturation of the rough endoplasmic reticulum and some brush border enzymes of mouse small intestine at the fetal stage (15-17 days) (150-152) in accordance with its suggested role as an inducer of enterocyte differentiation with a specific and restricted action. In suckling (8 days) animals however, injections of EGF stimulate proliferation in all gut segments, including small intestine, and simultaneously increase all brush border hydrolase activities in a dose-dependent manner (153). Therefore, EGF does not only act as a fetal promoting agent but seems to be one of the few factors involved in re-differentiation of epithelial cells during postnatal adaptation of intestinal function at weaning, a process which is highly coordinated along the entire digestive tract in rodents (147). Discrepancies were even observed between mouse and rat as exogenous EGF triggered limited effects on some brush border enzymic activities (154) or had no influence (155,156) in the latter species. In addition, EGF administered orogastrically but not intraperitoneally induced precocious maturation of intestinal disaccharidase activities in suckling rabbits (157). Many explanations can be put forward to explain these variations such as the amount of EGF used, route of administration, rapid uptake of exogenous EGF by the liver, phase of circadian rhythm, nutritional state, length of the experimental period, injection schedule and developmental stages as well as the possible masking effect of endogenous EGF ligands. Eventhough animal models are necessary for the identification of involved growth factors and characterization of their biological roles, it is clear that effects induced by EGF in rodent small intestine cannot be directly extrapolated to the human gut.

There are several evidences that EGF, TGFalpha and related substances may exert a similar influence on developing stomach of rodents (rat, rabbit, guinea pig) and humans (reviewed in 158). They stimulate epithelial proliferation and mucus synthesis while they act negatively on parietal cell differentiation. Moreover targeted overexpression of TGF-alpha gene in mice causes alterations of the gastric mucosa that ressemble Ménétrier's disease (46,159,160), a premalignant disorder characterized by foveolar hyperplasia, hypochlorhydia and increased mucus. Thus it appears that this hormonal system would assume a general and important role in development of the mucigenic gastric lineage. The earlier finding by Dembinski and Johnson (161) that EGF significantly increased weight of the whole stomach and DNA content of the oxyntic glands in unweaned rats but had no effect on pepsinogen synthesis/secretion led to the conclusion that EGF is a candidate inducer of oxyntic mucosal growth which does not participate in the developmental regulation of gastric zymogen expression. Our recent observation that this growth factor specifically influences gastric lipase expression in human chief cells (an enzyme absent in rodents) without affecting pepsinogen (140) stresses again the caution to be taken in directly extrapolating concepts established in animal models to humans.

6. INTEGRATION

In 1976, David Wingate formulated a new global theory of gastrointestinal hormone action, called the eupeptide system (162), which stated that the established model of endocrine function - the concept of endocrine glands secreting at a distance from target tissues - was inappropriate for the context of gut physiology. This theory proposed that local 'paracrine' polypeptides may be no less important and may cooperate with 'endocrine' true hormones or mediate some of their effects in the control of nutrient absorption, smooth muscle contraction and tissue maintenance. One must realized today that the discovery and the study of the EGF/EGF-R system has greatly contributed to the conceptual enlightenment of the former theory. As suggested by the variety of its biological actions and its widespread expression in developing and adult gut segments, this growth factor system seems to be involved in the global and intrinsic control of gut morphogenesis and homeostasis.

The current review emphasized on analyzing the expression of EGF-R in human fetal gut and correlating the biological actions of EGF-R ligands. The data presented reveal the ubiquitous distribution of the receptor in the developing mucosa of gut segments as well as in the various epithelial cell types. In comparison, its expression is known to become more restricted in the corresponding adult tissues. We may therefore propose an important and pleiotropic role for the EGF-R and its ligands in the context of gut epithelium development. Firstly, the growth factor system appears to be involved in the local regulation of epithelial cell proliferation in which EGF-R are abundant. Depending upon the specific segment and its differentiation status, cell proliferation would be stimulated or decreased by agonists of EGF-R; the growth factor acts as a mitogen for gastric explants and as an inducer of precocious maturation in small intestine (lactase activity increased) and colon (intestinal enzymes down-regulated). A second function of this system *in utero* may be to counter-regulate the terminal differentiation of human digestive epithelia; it negatively modulates the expression of segment-specific functional markers such as brush border sucrase, trehalase and glucoamylase in the intestine and chief cell lipase in the stomach. In the latter organ, the relative abundance of EGF-R in surface epithelium and the effect of EGF supplementation in culture also argues for a role in mucus synthesis. In addition, this hormonal system is involved in the normal maintenance of adult gut tissues (plus inhibition of gastric acid secretion) and it is deregulated in cancer (148,149). These facts illustrate that EGF-R and EGF-related factors still exert significant developmental activities in the more mature digestive tract.

According to the literature, multiple ligands for the EGF-R are present in tissues, including fetal organs. What would be the relevance of such a heterogeneity in the context of gut epithelium development and maintenance? Probably to serve in different delivery pathways or in different physiological responses (see figure 4). TGFalpha, for example, likely represents the fetal and paracrine ligand, which is synthesized in proliferative compartments of human fetal gut (123,163,164) and which locally regulates epithelial proliferation/differentiation. This factor activates EGF-R and mitogenesis as potently as EGF *in vitro* (165). It might be of some significance in this context that TGFalpha acts more strongly than EGF on cell proliferation, motility and branching morphogenesis in specific experimental systems (166 plus several studies cited in 148,149) due to differences in ligand

processing, receptor down-regulation and, possibly, binding efficiency. The global expression of both TGFalpha and cognate EGF-R are simultaneously down-regulated in maturing organs while EGF expression increases at the end of gestation and experiments realized in mouse provided evidences that these developmental changes are under the control of thyroid hormones (167,168). However, expression of the TGFalpha protein persists in a number of mature tissues and recent data demonstrate that it represents the main form locally produced in proliferative compartments of the adult gastrointestinal tract e.g. intestinal crypts and gastric glands (123,169-171). There are new evidences that HB-EGF, another agonist of EGF-R, might be the autocrine form expressed by epithelial cells in response to environmental factors. It is expressed together with EGF or TGFalpha in many tissues (172) and it is rapidly up-regulated by EGF-related ligands themselves (173) and by *Helicobacter pylori* in gastric cells (174). Its expression greatly increases in gut-associated carcinomas (175,176). HB-EGF thus represents an immediate early gene candidate. Also at the specific level of the human stomach, HB-EGF appears to be the main form produced by fundic parietal cells and gastrin cells of pyloric glands (177). Concerning EGF, the prototypical growth factor, it likely represents the long-distance acting form released into the gut lumen (a lumone) and one current hypothesis is that it would assume a surveillance role by maintaining the integrity of gut tissues upon mucosal damage (stimulation of epithelial restitution and proliferation)(178,179). It is interesting to remind, in this regard, earlier data from Wright and collaborators (180) showing that ulceration of the mucosal epithelium in the human gastrointestinal tract induced the development of a novel cell lineage that produced neutral mucin and abundant immunoreactive EGF. It is true that EGF secreted into the lumen is processed into smaller and less active forms (181,182) and that its stability is positively influenced by ingestion of food proteins (183,184). In the specific context of gut epithelial development in the human fetus and infant, it should be pointed out that EGF may remain bioactive for long periods in gastric and intestinal fluids due to the immaturity of pancreatic enzymic function (185) and the protective effect of milk proteins (186). However, the biological significance and efficiency of luminal binding on epithelial cells (especially in immature gut), the controversial identification of low- or high-affinity binding sites on apical membranes as well as the involvement of a transcytosis mechanism (serosal towards luminal or luminal towards serosal) remain to be clarified (148,187).

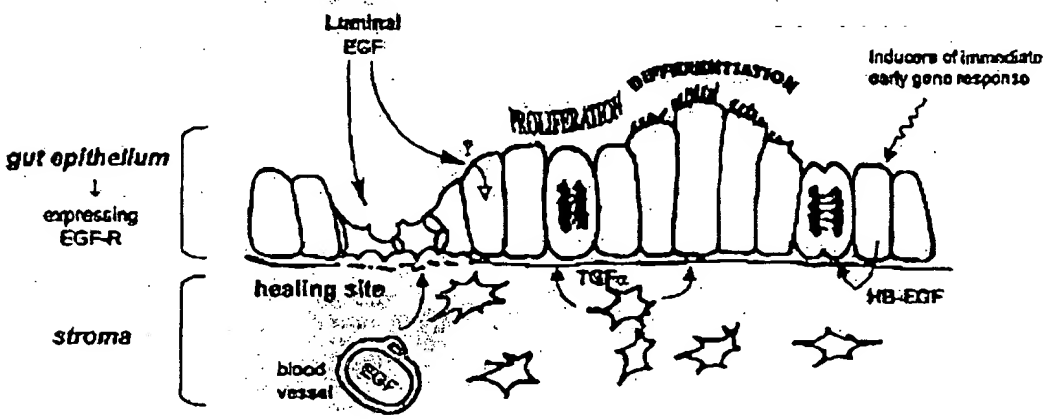


Figure 4. Theoretical model of EGF action on the EGF-R expressing gut epithelum.

EGF-related substances (EGF, TGFalpha, HB-EGF) derived from various sources would access to EGF-R at sites of epithelial damage or would be released in a paracrine/autocrine fashion at the epithelio-mesenchymal interface to participate in normal regeneration and differentiation.

7. PERSPECTIVES

The overall characteristics of EGF/EGF-R physiology in the developing human gastrointestinal tract do not entirely correlate with those found in animal models. The expression of the receptor or its ligands may also be altered or subjected to abnormal regulatory events in adenocarcinoma cell lines. For these reasons, it will be necessary to establish normal human epithelial cell models in order to further our understanding of the specific developmental actions of this growth factor system and its modulation during successive stages of epithelial differentiation.

Concerning the molecular properties of EGF-R activation and regulation, which are determined by the level of EGF-R expression and modulated by intracellular kinases, it now appears that they depend upon heterodimerization events with EGF-R-related receptors expressed in epithelial cells. HER-2, HER-3 and HER-4 (erbB2, erbB3 and erbB4 in mouse) are known to bind neuregulin-like substances and differential heterodimerization of EGF-R with each partner receptor might account for distinct responses (stimulatory or inhibitory) observed in given cell types (188,189). Interestingly, these EGF-R-related proteins seem to be abundantly expressed in human fetal gut compared to the adult (190,191) and some evidence for their dynamic expression in relation with epithelial morphogenesis was recently reported (192). At the light of experiments exploring MAP kinase activation kinetics in the presence of growth factors alone or growth factors with integrin ligands (87), it is now obvious that EGF-R can also synergize with receptors to extracellular matrix proteins for inducing an optimal response. Confirming this theory, the growth of human colon carcinoma cells was differentially stimulated by TGFalpha on plastic and collagen substratum while the induced-formation of crypt-like structures in 3-dimensional culture increased the expression of specific integrins (193). Future studies must be oriented to enlighten the intracellular events leading to the biological effects and to characterize the molecular events underlying the synergism between EGF-R and integrin ligands specific to human gastrointestinal epithelial cells.

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